The role of protein synthesis in regulation of oestradiol-17 β in the pro-oestrous hamster*

S. K. Saidapur† and G. S. Greenwald

*Department of Physiology, Ralph L. Smith Research Center,
University of Kansas Medical Center, Kansas City, Kansas 66103, U.S.A.*

Summary. Injection (s.c.) of 2 mg cycloheximide at 14:00 h on the day of pro-oestrus prevented the normal rise in serum progesterone and significantly lowered progesterone levels at 15:00 h. Values then rose but only to approximately half of the control values between 16:00 h and 19:00 h. Oestradiol levels also decreased drastically by 15:00 h but were significantly higher in cycloheximide-treated animals until 19:00 h. FSH and LH concentrations were not affected when cycloheximide was given at 14:00 h but treatment at 10:00 h resulted in generally lower values. Animals treated with cycloheximide at 14:00 h failed to ovulate (N = 9), but simultaneous injection of 50 μ g progesterone restored ovulation in 50% of the treated animals. In contrast, hamsters injected with cycloheximide at 10:00 h ovulated the next morning, suggesting that protein synthesis essential for ovulation is limited to the first 4–5 h after the release of LH.

Introduction

Previous in-vivo and in-vitro studies on the pro-oestrous rat (Hori, Ide & Miyake, 1969; Lieberman *et al.*, 1975; Hillensjö, Bauminger & Ahrén, 1976; Katz & Armstrong, 1976) and sheep (Moor, 1974) suggest that LH inhibits C-17:20 side chain cleavage and/or C-19 androgen-aromatase activity, leading to a rapid decline in oestrogen production. In hamsters, following the LH surge on the day of pro-oestrus, there is also a change in the pattern of ovarian steroidogenesis, with an abrupt decline in serum oestrogen and testosterone and a concomitant increase in serum progesterone concentrations (Saidapur & Greenwald, 1978, 1979a). The role of LH and progesterone in controlling ovarian production of oestradiol-17 β in the hamster has been studied by in-vivo and in-vitro methods (Saidapur & Greenwald, 1979b).

The present work was undertaken to investigate the possible role of protein synthesis in the regulation of oestradiol in the pro-oestrous hamster by using cycloheximide which is an inhibitor of protein synthesis. In addition, the effect of cycloheximide on ovulation was also studied.

Materials and Methods

Adult female hamsters (*Mesocricetus auratus*) weighing 80–120 g and maintained on a 14 h : 10 h dark schedule (lights on 07:00–19:00 h) were used after 3 or 4 consecutive 4-day cycles. Day 1 is defined as the day of ovulation. In all experiments hamsters were used on the day of pro-oestrus (Day 4). Cycloheximide (Sigma Chemical Co., St Louis, Missouri, U.S.A.) was dissolved in

* Reprint requests to Professor G. S. Greenwald.

† Present address: Department of Zoology, Karnatak University, Dharwad 580 003, India.

saline (9 g NaCl/l). Each s.c. injection was of 2 mg cycloheximide/0.2 ml saline. Control animals received only the 0.2 ml saline. Peanut oil was used to dissolve progesterone and a volume of 0.1 ml was injected s.c. At the end of the experiments hamsters were decapitated and trunk blood was saved for hormone assays.

Experiments

Experiment 1. The acute effects of 2 mg cycloheximide injected on the afternoon of pro-oestrus were investigated. Animals were injected at 14:00 h on Day 4 and some were killed every hour until 21:00 h. Serum was saved for assay of progesterone, oestradiol, LH and FSH concentrations.

Experiment 2. The acute effects of 2 mg cycloheximide given on the morning of pro-oestrus at 10:00 h were studied. Animals were killed at 11:00, 12:00 or 13:00 h and serum oestradiol, LH and FSH concentrations were measured.

Experiment 3. The effect of cycloheximide on ovulation was investigated by injecting 2 mg cycloheximide at 10:00 or 14:00 h on Day 4 and killing the animals the next day. Ova were flushed from the oviducts and counted under a dissecting microscope. Because the animals injected with cycloheximide at 14:00 h failed to ovulate, attempts were made to restore ovulation by simultaneously administering 50 µg progesterone (s.c.).

Assays

Details of the radioimmunoassays for progesterone and oestradiol have been described by Saidapur & Greenwald (1978) and Terranova & Greenwald (1978). The antisera used were specific for oestradiol-17β (Exley, Johnson & Dean, 1971) and progesterone (Surve, Bacso, Brinckerhoff & Kirsch, 1976). The lower limit of sensitivity was 2 pg oestradiol/tube and 5 pg progesterone/tube. Serum from chronically hypophysectomized–ovariectomized hamsters contained approximately 12 pg oestradiol/ml and 0.6 ng progesterone/ml.

The kits (rat) provided by the NIAMDD were used to determine serum FSH values. Serum LH was determined by the ovine–ovine RIA system of Niswender, Reichert, Midgely & Nalbandov (1971). The methodology and validation of the radioimmunoassays for the hamster have been previously documented (Bast & Greenwald, 1974) and details of the assays are given by Siegel, Bast & Greenwald (1976). NIAMDD-anti-rat FSH serum-7 was used in the FSH assay. Anti-ovine LH serum (GDN-15) and an ovine LH for iodination (LER-1056-C2) were kindly provided by Dr G. D. Niswender and Dr L. E. Reichert, respectively. Serum peptide levels were expressed in terms of NIAMDD-rat-FSH-RP1 ($2.1 \times \text{NIH-FSH-S1}$) and NIAMDD-rat-LH-RP1 ($0.03 \times \text{NIH-LH-S1}$).

Statistical analysis

In all experiments, comparisons with the means of the control group were made by the Student's *t* test, and values with $P < 0.05$ were considered statistically significant.

Results

Experiment 1

Injection of 2 mg cycloheximide at 14:00 h on the day of pro-oestrus not only prevented the normal rise in serum progesterone but also significantly lowered the levels within 1 h. Subsequently, there was a rise in serum progesterone between 16:00 and 19:00 h. The profile of serum progesterone was similar to that in controls, but the levels in the treated animals were

always approximately half of the control values (15:00–19:00 h) (Text-fig. 1a). Serum oestradiol levels also decreased drastically within 1 h of injection but then increased significantly ($P < 0.05$) over the control values (Text-fig. 1b) and remained higher than in controls until 19:00 h. By 21:00 h values of oestradiol in both groups were the same.

There was no difference in serum LH between the treated and the control groups except at 19:00 h when LH was higher in the treated animals (Text-fig. 1c). FSH was higher in the treated than in the control group only at 17:00 h (Text-fig. 1d).

Text-fig. 1. Serum concentrations (mean $\pm$ s.e.m. for 6–8 hamsters/group) of (a) progesterone, (b) oestradiol-17 β , (c) LH and (d) FSH at different times after treatment with 2 mg cycloheximide s.c. at 14:00 h on the day of pro-oestrus.

This experiment was repeated with hamsters obtained from a different breeding colony and at a different time of the year. A dose of 4 mg cycloheximide injected s.c. at 14:00 h on the day of pro-oestrus resulted the next morning (corresponding to Day 1 of the next cycle) in the ovulation of only one egg from a total of 6 treated hamsters. An additional 6 hamsters injected with cycloheximide at 14:00 h on the day of pro-oestrus all showed the characteristic post-ovulatory vaginal discharge at 09:00 h 4 days later and ovulated an average of 15.5 ± 0.76 eggs, still embedded in granulosa cells. Moreover, in addition to new rupture points the ovaries contained blood-filled follicular cysts representing unruptured follicles from the previous cycle.

The s.c. injection of cycloheximide was not associated with any acute or chronic signs of debilitation, e.g. changes in behaviour or loss of body weight. The hamsters used in the chronic study weighed 117.7 ± 6.5 g (s.e.m.) just before the injection of cycloheximide and 4 days later weighed 117.5 ± 4.9 g.

Experiment 2

Injection of 2 mg cycloheximide on the morning of Day 4 significantly depressed serum oestradiol within 1 h and this decline continued until 13:00 h (Table 1). LH levels increased during the first hour after treatment and returned to control values by the next hour, decreasing significantly by 13:00 h. Serum FSH values were significantly ($P < 0.05$) lower at 11:00 and 12:00 h but returned to control values by 13:00 h (Table 1).

Table 1. Effect of cycloheximide given at 10:00 h on mean $\pm$ s.e.m. serum hormone concentrations in pro-oestrous hamsters (no. in parentheses)

Treatment	Hormone conc. at:		
	11:00 h	12:00 h	13:00 h
Oestradiol-17 β (pg/ml)			
Control	134 $\pm$ 11 (12)	117 $\pm$ 6 (12)	94 $\pm$ 10 (6)
Cycloheximide	*38 $\pm$ 3 (6)	*56 $\pm$ 7 (6)	*55 $\pm$ 8 (6)
LH (ng/ml)			
Control	28 $\pm$ 6 (5)	33 $\pm$ 7 (6)	31 $\pm$ 3 (6)
Cycloheximide	*48 $\pm$ 4 (6)	33 $\pm$ 3 (6)	*13 $\pm$ 2 (5)
FSH (ng/ml)			
Control	95 $\pm$ 6 (12)	99 $\pm$ 10 (13)	63 $\pm$ 6 (6)
Cycloheximide	*61 $\pm$ 9 (6)	*44 $\pm$ 3 (6)	50 $\pm$ 7 (5)

* Significantly different from control values, $P < 0.05$ (Student's *t* test).

Experiment 3

Animals injected with cycloheximide at 10:00 h on the day of pro-oestrus ovulated the next morning an average of 12.4 ± 0.8 eggs ($N = 5$). In contrast, the same treatment at 14:00 h blocked ovulation in all animals ($N = 9$). Although there was a vaginal discharge typical of the normal post-ovulatory event, ovarian histology revealed the presence of atretic Graafian follicles in these animals. Administration of 50 μ g progesterone with the cycloheximide at 14:00 h restored ovulation in some of the animals (4/8 ovulated 12, 11, 9 and 8 eggs, respectively).

Discussion

The present work shows that inhibition of protein synthesis by cycloheximide results in changes in serum progesterone and oestradiol in the pro-oestrous hamster (Text-figs 1a and 1b; Table 1) suggesting, albeit indirectly, the involvement of protein synthesis in the regulation of ovarian steroidogenesis. The effects on oestradiol concentrations of cycloheximide on the afternoon of pro-oestrus were not mediated through changes in serum levels of gonadotrophins (Text-figs 1c and 1d). Cycloheximide may therefore act directly at the ovarian level as suggested by Alleva, Bonventre & Lamanna (1979); i.e. cycloheximide inhibits the synthesis of lytic enzymes needed for ovulation rather than enzymes involved in steroidogenesis. These authors did not measure peripheral progesterone or oestradiol concentrations but normal progesterone or oestradiol concentrations were presumed because behavioural oestrus was unaffected. In the present study, the fact that progesterone levels were halved as a consequence of cycloheximide administration indicates that inhibition of protein synthesis also affects enzymes involved in steroidogenesis. Reconciling these results with those of Alleva *et al.* (1979), it is likely that reduced progesterone

levels are still sufficient to evoke behavioural oestrus but not ovulation. This supposition is further strengthened by the fact that simultaneous administration of 50 µg progesterone restored ovulation in 50% of the animals treated with cycloheximide.

In the present study cycloheximide was injected subcutaneously. Alleva *et al.* (1979) injected the drug intraperitoneally and the lowest dose of cycloheximide needed to block ovulation was 6 mg, with 6/15 hamsters dying within 1–6 days. In our experiment, 2–4 mg cycloheximide inhibited ovulation without any mortality and without disturbing the normal 4-day duration of the next oestrous cycle. Obviously the subcutaneous injection of cycloheximide causes only a transitory and a relatively mild depression in protein synthesis. The problems inherent in the in-vivo administration of inhibitors of protein synthesis were circumvented in the study of Lieberman *et al.* (1975) in which explanted rat Graafian follicles were exposed to LH: addition of puromycin 1–3 h later inhibited progesterone accumulation within $\frac{1}{2}$ –1 h.

Rondell (1974) proposed that ovulation induced by LH involves two stages: LH-induced steroidogenesis from acetate or cholesterol to progesterone, and progesterone-induced synthesis of collagenase (or other lytic enzymes) in the ovarian follicle wall, causing its dissolution and ultimate ovulation. In the present study, cycloheximide inhibited ovulation when injected on the afternoon of pro-oestrus but not when the inhibitor was injected at 10:00 h (4–5 h before the LH surge). Previous authors have suggested that protein synthesis essential for ovulation is restricted to the first 4–5 h after LH release (Pool & Lipner, 1964, 1966; Alleva *et al.*, 1979), and the present observations are in agreement with this view.

The injection of cycloheximide at 10:00 h on the day of pro-oestrus depressed serum oestradiol drastically over the 3-h period studied (Table 1). The injection of progesterone at 10:00 h to pro-oestrous hamsters yielded similar results (Saidapur & Greenwald, 1979b). It is difficult to explain the similar effects of progesterone and cycloheximide since the former induces while the latter inhibits protein synthesis. Cycloheximide may block synthesis of the enzymes involved in steroidogenesis while progesterone may induce the formation/synthesis of an inhibitor protein that interferes with steroidogenesis of androgens and/or oestrogens.

This view is initially difficult to reconcile with the results obtained after injecting cycloheximide on the afternoon of pro-oestrus. Oestradiol levels declined significantly during the first hour, as they did after the morning injection (Text-fig. 1b), but then they abruptly increased at 16:00 h, presumably in response to the normal LH and FSH surges (Text-figs 1c and d). However, the levels of progesterone did not rise to the same extent as in controls (Text-fig. 1a) despite the normal LH surge. If cycloheximide were blocking the enzymes involved in steroidogenesis at some point earlier than the synthesis of progesterone, progesterone synthesis would be expected to be low, as seen in the present study (Text-fig. 1a). The low levels of progesterone might not be enough to bring about a decline in the secretion of oestradiol, thus leading to an elevated oestrogen level for a prolonged period (Text-fig. 1b). The maintained high FSH levels at 17:00 h (Text-fig. 1c) in cycloheximide-treated animals could also facilitate the aromatization of testosterone to oestradiol.

From the results of our earlier and present studies it is clear that complex mechanisms involving the interaction of LH, progesterone and protein synthesis are responsible for the change in the pattern of ovarian steroidogenesis on the afternoon of pro-oestrus. Whether these changes involve granulosa or thecal cells or both is undetermined. Moreover, differences in the regulation of progesterone synthesis by the follicle and interstitium (Saidapur & Greenwald, 1979a) may account for the different effects of cycloheximide on circulating levels of oestradiol when given during the morning or afternoon of pro-oestrus. Obviously, more research is needed for a better understanding of this phenomenon.

The research was supported by grants from NIH (HD-00596) and the Ford Foundation. S.K.S. was supported as a Ford Foundation Postdoctoral Fellow in reproductive physiology.

References

- Alleva, J.J., Bonventre, P.F. & Lamanna, C. (1979) Inhibition of ovulation in hamsters by the protein synthesis inhibitors diphtheria toxin and cycloheximide (40639). *Proc. Soc. exp. Biol. Med.* **162**, 170–174.
- Bast, J.D. & Greenwald, G.S. (1974) Serum profiles of follicle-stimulating hormone, luteinizing hormone and prolactin during the estrous cycle of the hamster. *Endocrinology* **94**, 1295–1299.
- Exley, D., Johnson, M.W. & Dean, P.D.G. (1971) Antisera highly specific for 17β -estradiol. *Steroids* **18**, 605–620.
- Hillensjö, T., Bauminger, S. & Åhrén, K. (1976) Effect of luteinizing hormone on the pattern of steroid production by preovulatory follicles of pregnant mares serum gonadotrophin-injected immature rats. *Endocrinology* **99**, 996–1002.
- Hori, T., Ide, M. & Miyake, T. (1969) Pituitary regulation of preovulatory oestrogen secretion in the rat. *Endocr. Japon.* **16**, 351–360.
- Katz, Y. & Armstrong, D.T. (1976) Inhibition of ovarian estradiol- 17β secretion by luteinizing hormone in prepubertal, pregnant mare serum treated rats. *Endocrinology* **99**, 1442–1447.
- Lieberman, M.E., Barnea, A., Bauminger, S., Tsafirri, A., Collins, W.P. & Lindner, H.R. (1975) LH effect on the pattern of steroidogenesis in cultured Graafian follicles of the rat: dependence of macromolecular synthesis. *Endocrinology* **96**, 1533–1542.
- Moor, R.M. (1974) The ovarian follicle of the sheep: inhibition of oestrogen secretion by luteinizing hormone. *J. Endocr.* **61**, 455–463.
- Niswender, G.D., Reichert, L.E., Midgely, A.R., Jr & Nalbandov, A.V. (1971) Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology* **84**, 1166–1173.
- Pool, W.R. & Lipner, H. (1964) Inhibition of ovulation in the rabbit by actinomycin D. *Nature, Lond.* **203**, 1385–1387.
- Pool, W.R. & Lipner, H. (1966) Inhibition of ovulation by antibiotics. *Endocrinology* **79**, 858–864.
- Rondell, P. (1974) Role of steroid synthesis in the process of ovulation. *Biol. Reprod.* **10**, 199–215.
- Saidapur, S.K. & Greenwald, G.S. (1978) Peripheral blood and ovarian levels of sex steroids in the cyclic hamster. *Biol. Reprod.* **18**, 401–408.
- Saidapur, S.K. & Greenwald, G.S. (1979a) Ovarian steroidogenesis in the proestrous hamster. *Biol. Reprod.* **20**, 226–234.
- Saidapur, S.K. & Greenwald, G.S. (1979b) Regulation of 17β -estradiol synthesis in the proestrous hamster: role of progesterone and luteinizing hormone. *Endocrinology* **105**, 1432–1439.
- Siegel, H., Bast, J.D. & Greenwald, G.S. (1976) The effects of phenobarbital and gonadal steroids on periovulatory serum levels of LH and FSH in the hamster. *Endocrinology* **98**, 48–55.
- Surve, A.H., Bacso, I., Brinckerhoff, J.H. & Kirsch, S.J. (1976) Plasma levels of progesterone in pseudo-pregnant rabbits actively immunized with a progesterone-protein conjugate. *Biol. Reprod.* **15**, 343–349.
- Terranova, P.F. & Greenwald, G.S. (1978) Steroid and gonadotrophin levels during the luteal-follicular shift of the cyclic hamster. *Biol. Reprod.* **18**, 170–175.

Received 4 July 1980